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PB-295 807

Neoplasia in Soft-Shell Clams (Mya Arenaria) Collected from Oil-Impacted Sites

(U.S.) Environmental Research Lab, Narragansett, RI

Sep 77

TECHNICAL REPORT DATA
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1. REPORT NO. EPA-600/J-77-152	2. JOURNAL ARTICLE
3.	PB 295807
4. TITLE AND SUBTITLE Neoplasia in Soft-Shell Clams (Mya Arenaria) Collected from Oil-Impacted Sites	5. REPORT DATE 6. PERFORMING ORGANIZATION CODE
7. AUTHOR(S) Paul P. Yevich and Carolyn A. Barszcz	8. PERFORMING ORGANIZATION REPORT NO.
9. PERFORMING ORGANIZATION NAME AND ADDRESS U.S. Environmental Protection Agency Environmental Research Laboratory Narragansett, RI 02882	10. PROGRAM ELEMENT NO. 11. CONTRACT/GRANT NO.
12. SPONSORING AGENCY NAME AND ADDRESS Same as above	13. TYPE OF REPORT AND PERIOD COVERED journal reprint 14. SPONSORING AGENCY CODE EPA/600/05

15. SUPPLEMENTARY NOTES

REFERENCE: ANNALS OF THE NEW YORK ACADEMY OF SCIENCES, 298:409-426, September 29, 1977

16. ABSTRACT

The Histopathology Unit of our laboratory has been involved for the past 7 years in the histopathologic examination of marine invertebrates collected from various oil spill sites along the coastal areas of the United States (TABLE 1). Of the 18 species of marine invertebrates examined from 11 oil spill sites, only soft-shell clams, *Mya arenaria*, collected from two oil spill sites, Long Cove, Searsport, and Harpswell Neck in Maine (FIGURES 1 & 2), have consistently shown neoplasms. The histopathologic results of the Long Cove surveys performed prior to 1975 were reported by Barry and Yevich.[1] Information on the ecologic and chemical evaluation of the Long Cove area is available in papers by Dow and Hurst[2] and Mayo *et al.* [3] No ecologic and chemical evaluation was performed on the Harpswell Neck area.

17. KEY WORDS AND DOCUMENT ANALYSIS

a. DESCRIPTORS	b. IDENTIFIERS/OPEN ENDED TERMS	c. COSATI Field/Group
Aquatic Biology, Marine Biology, Water Pollution, Oil Pollution, Aquatic animals, Mollusca, Bivalves, Oil, Cancer, Neoplasms, Histopathology, Pathology		

18. DISTRIBUTION STATEMENT Release Unlimited	19. SECURITY CLASS *(This Report)*	21. NO. OF PAGES 18 20
	20. SECURITY CLASS *(This page)*	22. PRICE A02-A01

EPA Form 2220-1 (9-73)

EPA-600/J-77-152
JOURNAL ARTICLE

Reprinted from
ANNALS OF THE NEW YORK ACADEMY OF SCIENCES
Volume 298 Pages 409–426
September 29, 1977 25386

NEOPLASIA IN SOFT-SHELL CLAMS (*MYA ARENARIA*) COLLECTED FROM OIL-IMPACTED SITES

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The Histopathology Unit of our laboratory has been involved for the past 7 years in the histopathologic examination of marine invertebrates collected from various oil spill sites along the coastal areas of the United States (TABLE 1). Of the 18 species of marine invertebrates examined from 11 oil spill sites, only soft-shell clams, *Mya arenaria*, collected from two oil spill sites, Long Cove, Searsport, and Harpswell Neck in Maine (FIGURES 1 & 2), have consistently shown neoplasms. The histopathologic results of the Long Cove surveys performed prior to 1975 were reported by Barry and Yevich.[1] Information on the ecologic and chemical evaluation of the Long Cove area is available in papers by Dow and Hurst[2] and Mayo *et al.*[3] No ecologic and chemical evaluation was performed on the Harpswell Neck area.

MATERIALS AND METHODS

The soft-shell clams collected from the oil spill sites in Maine were processed as previously described by Barry and Yevich[1] by use of Helly's fixative. Helly's fixative became the fixative of choice for marine mollusks because studies by Barszcz and Yevich[4] have shown that it is superior to several other commonly used fixatives in preserving cellular detail, especially cytoplasmic granules.

RESULTS

Gross Examination

At no time during the gross examination of the soft-shell clams from either Long Cove or Harpswell Neck were any abnormalities noted.

Microscopic Examination

Long Cove, Maine

During the past 5 years, neoplasia of the reproductive tract was found in 1–22% of the soft-shell clams collected from stations in Long Cove, with the following exception. Animals collected in late 1974 and in 1975 from station 5 (1) had neoplastic changes in the connective tissue areas of the body, the hematopoietic system, rather than gonadal neoplasia. This hematopoietic neoplasm will be described in the Harpswell Neck results. TABLE 2 gives the results of the 1974–75 surveys at Long Cove. Asterisks indicate the animals found to have hematopoietic neoplasms.

Examination of the soft-shell clams over the 5-year period showed various stages

TABLE I

OIL SPILL SITES STUDIED

Site of Spill	Year of Spill	Type of Oil	Species Examined Histopathologically
West Falmouth, Massachusetts	1970	no. 2 fuel oil	scallops (*Aequipecten irradians*)
Cousins River, Maine	1970	no. 2 fuel oil	soft-shell clams (*Mya arenaria*)
Bridgeport, Connecticut	1970	no. 2 fuel oil	oysters (*Crassostrea virginica*)
New Haven, Connecticut	1971	no. 2 fuel oil	quahogs (*Mercenaria mercenaria*) lobsters (*Homarus americanus*)
Searsport, Maine	1971	no. 2 fuel oil & JP-5	soft-shells (*Mya arenaria*)
Harpswell Neck, Maine	1972	JP-4	soft-shells (*Mya arenaria*)
Searsport, Maine	1972	no. 2 fuel oil & JP-5	soft-shells (*Mya arenaria*)
Anacortis, Washington	1972	diesel oil	mud snail (*Nassarius obsoletus*) butter clams (*Saxidomus giganteus*) little necks (*Protothaca staminea*) mussels (*Mytilus edulis*) oysters (*Crassostrea gigas*)
Casco Bay, Maine	1973	bunker C	lobsters (*Homarus americanus*) soft-shell clams (*Mya arenaria*)
Friendship Harbor, Long Island Maine	1973	bunker C	lobsters (*Homarus americanus*) soft-shell clams (*Mya arenaria*)
Searsport, Maine	1973	no. 2 fuel oil & JP-5	soft-shell clams (*Mya arenaria*) mussels (*Mytilus edulis*) clam worms (*Nereis virens*) blue crabs (*callinectes sapidus*)
Searsport, Maine	1974	no. 2 fuel oil & JP-5	soft-shell clams (*Mya arenaria*)
Canadian-American Border, Maine	1974	bunker C	soft-shell clams (*Mya arenaria*)
Searsport, Maine	1975	no. 2 fuel oil & JP-5	soft shell clams (*Mya arenaria*)
Harpswell Neck, Maine	1975	JP-4	soft-shell clams (*Mya arenaria*)
Florida Keys, Florida	1976	no. 2 fuel oil	corals (*Acropora palmata*) (*Manicina aerolata*) (*Porites divaricata*) (*Porites furcata*) (*Acropora cervicornis*) (*Millipora complanata*)

FIGURE 1. Map of Long Cove, Searsport, Maine, shows collection sites (1975 survey). Numbers in parentheses denote stations in previous studies.

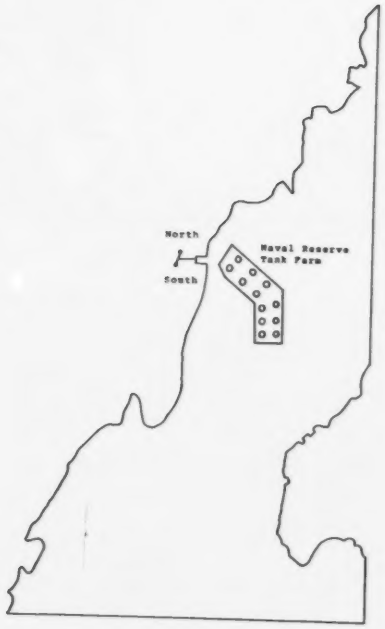

FIGURE 2. Map of Harpswell Neck, Maine, shows collection sites.

of neoplastic development in the reproductive tracts of both males and females. While studying the 182 animals with gonadal neoplastic lesions, the following pattern of histogenesis emerged. In the early stages of development, neoplastic cells were found along the follicular walls and within the follicular network. These cells were small and relatively uniform in size, resembling primitive germ cells, except that the cytoplasm was eosinophilic instead of basophilic. The nuclei were round or slightly irregular and at times had a pink-staining nucleolus. The chromatin, often seen in a thready pattern, was either clumped or finely distributed (FIGURE 3). At times, mitotic figures and multinucleated cells were noted.

In a few animals, only small foci of neoplastic cells that occupied small portions of follicles were noted, and the sex of the animals was readily determined by its gonadal characteristics (FIGURE 4). As the neoplastic cells increased in number, atypical cell inclusions or follicular cell inclusions and primitive germ cells were still seen within the follicles. Normal development of the oocytes or spermatogonia continued despite the inclusion of the neoplastic cells. Follicles free of neoplastic cells developed normally.

TABLE 2

INCIDENCE OF GONADAL NEOPLASMS IN SOFT-SHELL CLAMS, *Mya arenaria*, LONG COVE, SEARSPORT, MAINE

Station Number	Survey Number	Date	Number of Clams	Number of Tumors	Percentage of Tumors
1	14	10/75	100	13	13.0
2	14	10/75	100	12	12.0
3	14	10/75	100	12	12.0
4	14	10/75	100	1	1.0
5 (1)	12	12/74	81	3*	3.6
	13	6/75	104	0	0.0
	14	10/75	100	2*	2.0
6	14	10/75	100	3	3.0
7 (1a)	14	10/75	100	3	3.0
8 (1b)	14	10/75	100	3	3.0
9 (1c)	14	10/75	100	10	10.0
10	14	10/75	100	11	11.0

*Hematopoietic neoplasms.

In a large number of animals, most of the follicles were jammed with neoplastic cells, causing distension of the follicles and loss of the distinguishing sexual characterics (FIGURE 5). A thickening of the follicular wall by collagenous connective tissue was noted in many of the involved follicles (FIGURE 6). In some cases, follicles were in the process of degeneration. The centers of these degenerating follicles contained neoplastic cells in various stages of necrosis, phagocytic amebocytes with cellular debris, pink and yellowish granules, and giant cells (FIGURE 7).

Metastasis was noted in 73 of 182 (40%) of the clams that had gonadal neoplasms. The cell types at the metastatic sites were the same as those seen in the follicles, but more mitotic figures were present. At some of the sites, there was an increase in collagenous connective tissue and a granular amebocytic infiltration (inflammatory response), a condition especially noted in the gills. At times, the metastasis was so extensive that individual follicles could not be differentiated, and the muscle bundles were the only discernible histologic structure of the reproductive tract. At least one-

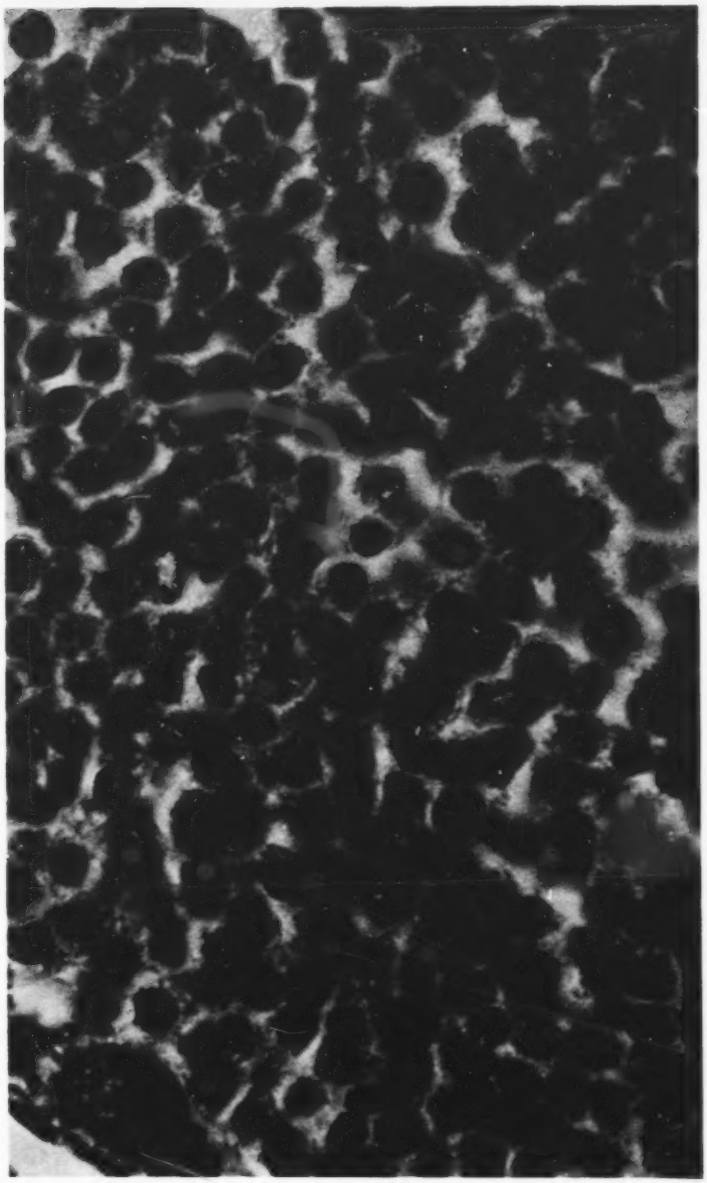

FIGURE 3. Female soft-shell clam, Long Cove. Neoplastic cells fill the follicle of the female reproductive tract. Note the oocyte in upper left corner. ×36.

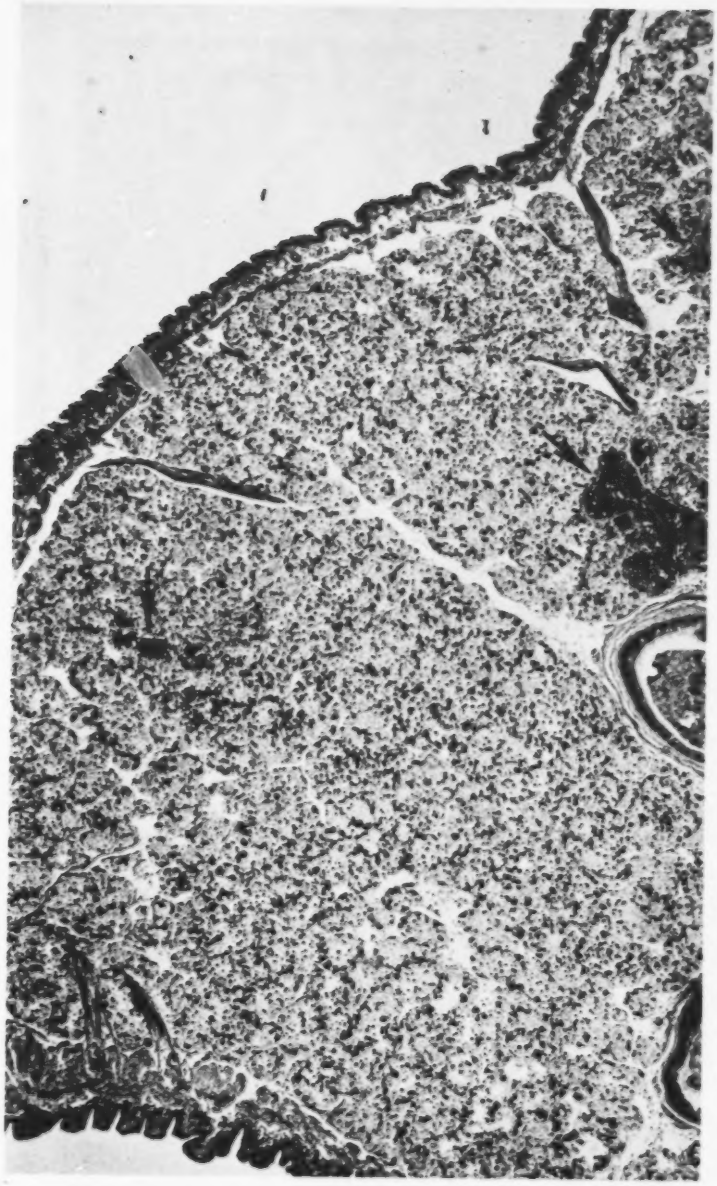

FIGURE 4. Female soft-shell clam, Long Cove. Arrows point to foci of neoplastic cells. Rest of the gonad is normal. ×2.5.

FIGURE 5. Soft-shell clam, sex unknown, Long Cove. Follicles are distended with neoplastic cells. ×5.7.

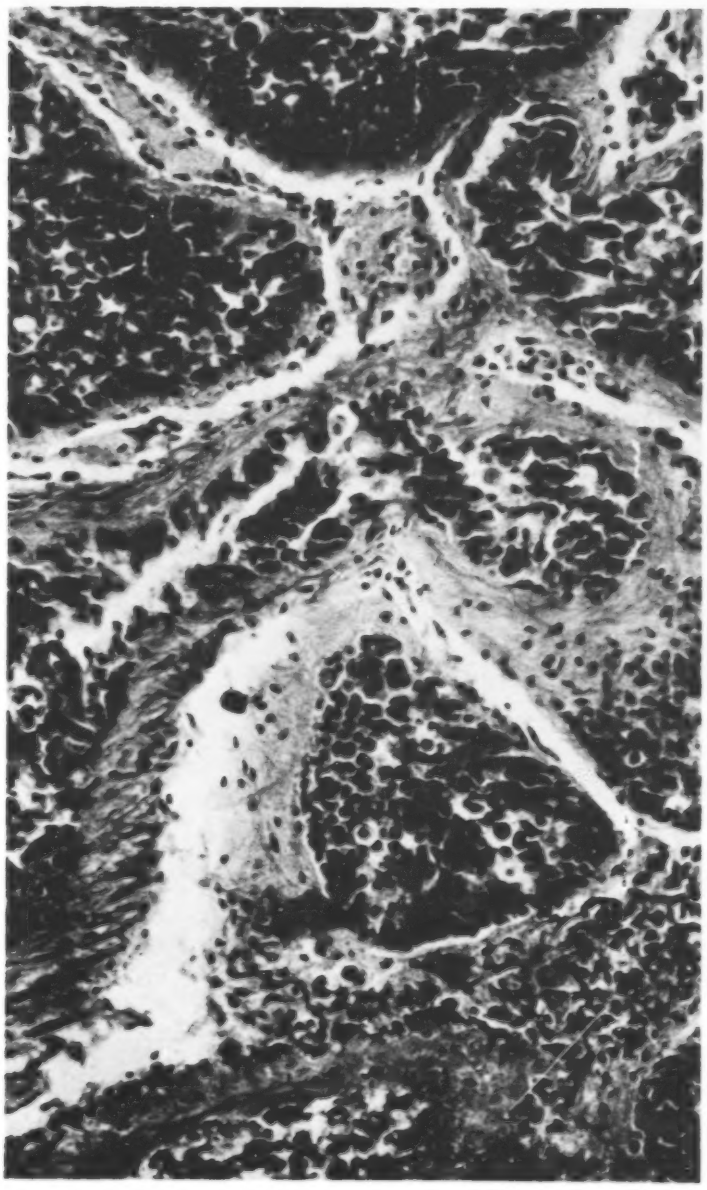

FIGURE 6. Soft-shell clam, sex unknown, Long Cove. Follicles and duct filled with neoplastic cells. Note thickened follicle wall. ×22.5.

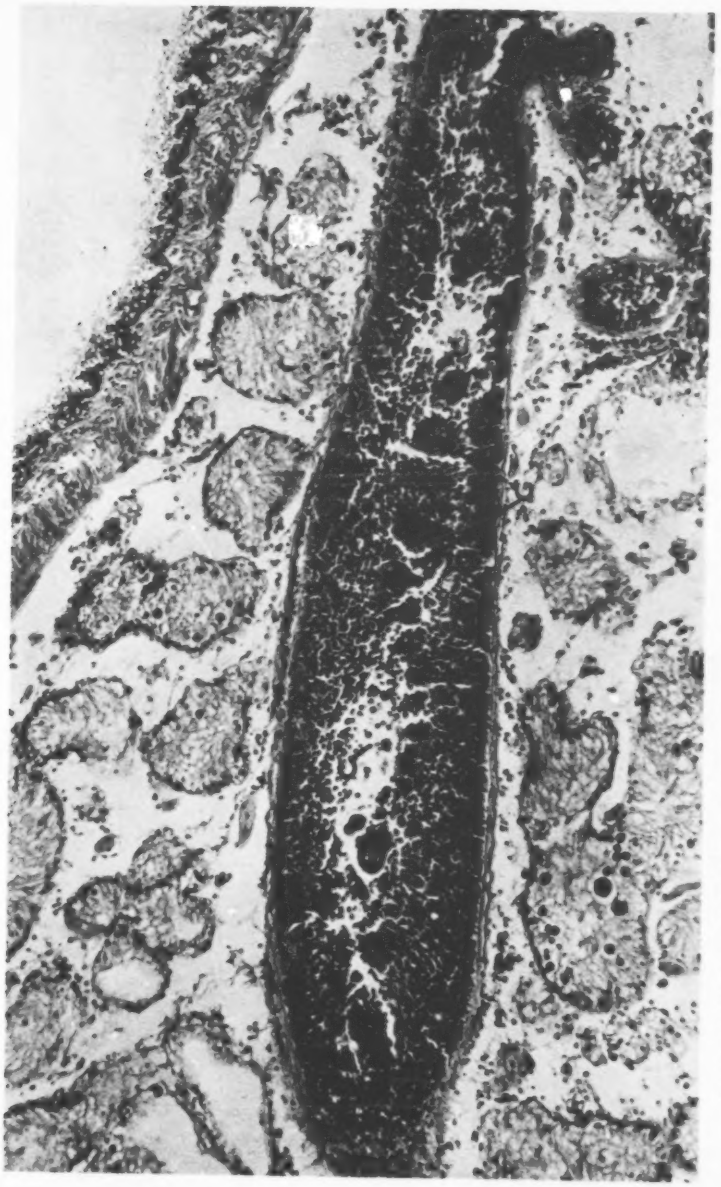

FIGURE 7. Female soft-shell clam, Long Cove. Gonadal duct with neoplastic cells in the process of degeneration. Note necrotic center with cell debris (C) and giant cells (G). Follicle with small foci of neoplastic development (N). Invasion of connective tissue between follicles (I). ×10.

FIGURE 8. Female soft-shell clam, Long Cove. Note extensive metastasis, one-third of the body occupied by the neoplastic cells. Metastasis extends to the gill arch (A) and the epibranchial wall (E). ×2.5.

third of the body of one animal was occupied by the neoplastic cells (FIGURE 8). Sites of metastasis were found in the walls of the epibranchial chamber, siphon, mantle, body wall, auricle, ventricle, pericardial wall, kidney, urinary pores, genital pore, and red gland, with the highest incidence being in the gills (FIGURE 9).

Foci of free neoplastic cells were noted in the sinuses of the visceral connective tissue and in the blood vessels of the gills (FIGURE 10). Invasion by the neoplastic cells was seen in the visceral connective tissue, genital pore, kidney, urinary pores, and body wall. TABLE 3 gives the numbers of animals examined each year, numbers and percentages of animals with neoplasms, numbers and percentages of animals with metastasis, and sites of metastasis, with the numbers of animals that had metastasis to each area mentioned.

Harpswell Neck, Maine

The neoplasms found in the soft-shell clams collected from Harpswell Neck, Maine differed from those seen in the majority of clams from Long Cove. In the Harpswell animals neoplastic cells of hematopoietic origin diffused throughout the connective tissue areas of the animals. These cells appeared to develop from the reticular connective tissue, with foci usually appearing first in the siphon or foot regions. The size of the cells varied; some had a small amount of cytoplasm that contained fine red granules and nuclei that were large, pleomorphic, or lobed with a large pink-staining nucleolus and a granular threadlike chromatin network. Other neoplastic cells had smaller hyperchromatic nuclei with an increase in the amount of cytoplasm and in the number of pink granules. Numerous mitotic figures were noted, as were cells in the process of degeneration and phagocytic amebocytes filled with large orange granules. These neoplastic cells were very invasive, at times causing destruction of the various organs and tissues (FIGURE 11). TABLE 4 shows the numbers and percentages of Harpswell Neck animals that had hematopoietic neoplasms.

DISCUSSION

From 1967 to the present time, our Histopathology Unit has examined more than 14,000 soft-shell clams, collected principally from coastal areas of Maine, Massachusetts, Rhode Island, Maryland, and California. Some of these animals were from clean areas, and some were from areas contaminated by oil, heavy metals, or other pollutants. No neoplasms were seen in soft-shelled clams from any areas other than Long Cove and Harpswell Neck, Maine.

In the neoplasms of the reproductive tract of the animals collected from Long Cove, many of the germinal cells continued to proliferate rather than mature, eventually taking over the whole follicle. These neoplastic cell invaded through the follicle wall into the surrounding connective tissue. Metastasis continued through the sinuses and blood vessels to other organs, where it developed and replaced the normal architecture of those organs. Thus, it is probable that there was interference with the physiologic functions of the animal. To use Dawe's[5] definition, we have a neoplasm "which endangers the continuation of the host's ecosystem." If these neoplasms are to be classified, they should be called "dysgerminomas" for females or "seminomas" for males. To use the clinical terms, they are "malignant" or "cancerous."

In the animals that had gonadal neoplasms, a surprisingly high incidence of

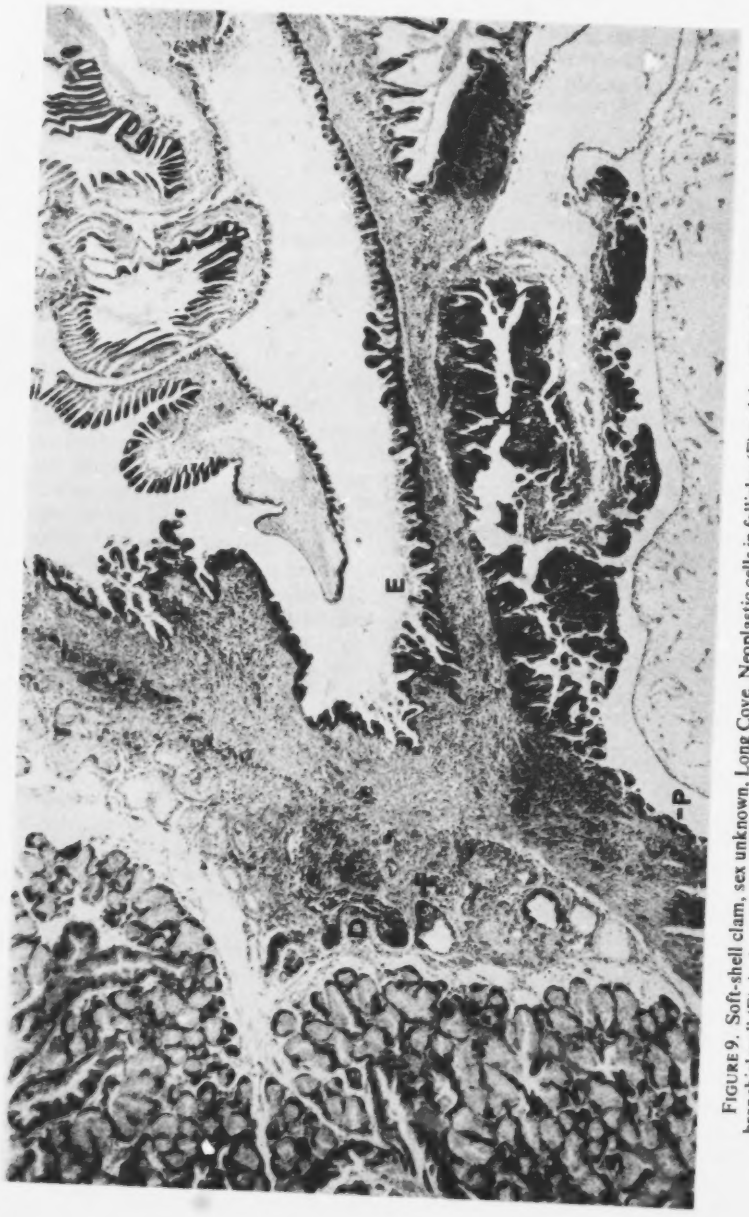

FIGURE 9. Soft-shell clam, sex unknown, Long Cove. Neoplastic cells in follicles (F) and duct (D). Note metastasis to the epibranchial wall (E), body wall (B), pericardial wall (P), kidney (K), and auricular wall (A). ×2.5.

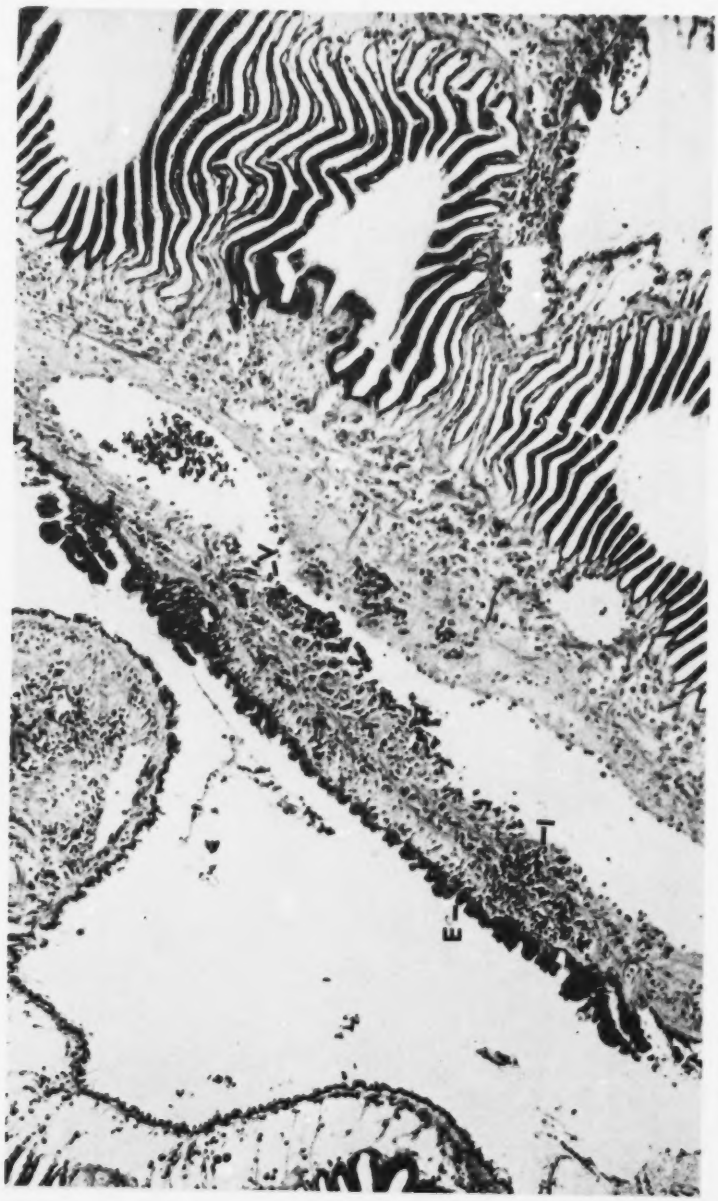

FIGURE 10. Soft-shell clam, sex unknown, Long Cove. Metastasis to the epibranchial wall (E) and the branchial vein (V). Note invasion of the blood vessel wall (I). ×5.7.

TABLE 3
INCIDENCE OF METASTASIS OF GONADAL TUMORS FOUND IN SOFT-SHELL CLAMS

Year	Number of Animals Examined	Number of Animals with Neoplasms	Number of Animals with Metastasis	Sites of Metastasis with Number of Clams with Metastasis to Each Site		
1971	1265	62 (4.1)*	28 (45)*	epibranchial chamber wall 8 genital pore 3 kidney 5 gills 20	urinary pore 2 snout 3 mantle 4 body wall 3	auricle 3 ventricle 1 pericardial wall 3 red gland 2
1972	398	29 (7)	7 (24)	gills 7 kidney 2 genital pore 2 urinary pore 2	mantle 1 pericardial wall 1 epibranchial chamber wall 2 body wall 2	
1973	200	13 (7)	3 (23)	gills 2 epibranchial chamber wall 1 kidney 1 body wall 1	genital pore 1	
1974	246	20 (8)	7 (35)	gills 4 auricle 2 pericardial wall 1 epibranchial chamber wall 2	kidney 2 body wall 1 ventricle 1 mouth 1	blood vessel wall 1
1975	1104	58 (5)	28 (48)	gills 15 kidney 3 body wall 6 epibranchial chamber wall 6	red gland 2 snout 2 genital pore 7 urinary pore 3	

*Numbers in parentheses are percent.

FIGURE 11. Visceral ganglion from soft-shell clam, sex unknown, Harpswell Neck. Note infiltration of the ganglion by the hematopoietic cells, with almost complete destruction of the normal architecture. Arrow points to the few remaining neurons. ×5.7.

metastasis was found, especially to the gills. The metastasis occurred via three routes, the first being by direct extension or invasion through the walls of the follicles and gonadal duct into the visceral connective tissue. In many mollusks, germinal epithelium is found in the gonadal duct right up to the genital pore, which lies next to the kidney and urinary pore and empties into the epibranchial chambers of the gills. When the gonadal duct is lined with neoplastic cells, there may be direct invasion into the genital pore, kidney, and urinary pore.

It is also possible for the neoplastic cells that are shed from the walls of the follicles and ducts to be released via the genital pore into the epibranchial chambers, water tubes, and ostia of the gills. The epibranchial chamber walls, walls of the water tubes, and ostia are normally lined with mucus-secreting cells. The exfoliated neoplastic cells may adhere to the mucus-secreting cells and from there colonize the water tubes, ostia, and epibranchial chambers of the gills.

The third route of metastasis is via the sinuses and blood vessels within the visceral mass, through which the neoplastic cells can move to other organs of the body. As shown in FIGURE 12, small foci of neoplastic cells are found in the blood vessels of the gill arches, with some cells lying free in the lumen and others attached to the

TABLE 4

INCIDENCE OF HEMATOPOIETIC NEOPLASMS IN SOFT-SHELL CLAMS, *Mya arenaria*, HARPSWELL NECK, MAINE

Station Number	Survey Number	Date	Number of Animals	Number of Tumors	Percentage of Tumors
South	1	5/25/72	31	6	19
North	1	5/25/72	27	5	18
South	2	9/17/72	31	7	22
North	2	9/17/72	41	5	1
South	3	6/20/75	132	4	3
North	3	6/20/75	105	6	5
South	4	8/ 6/75	104	15	14

vascular wall. In many of the metastatic sites in the gills, the capillaries in the filaments appear to be the only areas of involvement, and it may be assumed that the capillary bed of the gills trapped the neoplastic cells that had been shed into the blood sinuses. Because the area of oxygen exchange in the soft-shell clam is the capillaries of the gill filaments, their physiologic function was probably impaired by the presence of the neoplastic cells.

The hematopoietic neoplasms found in the animals collected from Harpswell Neck were very much like those described by Farley[6] in oysters, Farley[7] in clams, and Brown *et al.*[8] in clams. However, one of the differences noted was that the cytoplasm in the neoplastic cells of the animals from the Harpswell site contained pink-staining granules, much like those seen in the granular amebocytes of normal animals. We believe that the cells are atypical precursors of the granular amebocytes and not those of the hyaline hemocytes mentioned by Farley.[6] Many theories of blood formation stress the mesenchymal cell or reticular cell as being the precursor of the blood cells.[9] Our observations indicate that these neoplastic cells arise from the fixed mesenchymal cells, reticular cells, of the connective tissue of these animals.

FIGURE 12. Female soft-shell clam, Harpswell Neck. Hematopoietic tumor. Note infiltration of the connective tissue between the follicles of the reproductive tract and the tubules and ducts of the digestive diverticula by neoplastic cells. ×22.5.

ETIOLOGY

There was no relationship between sex or stage of sexual development of the animals and the presence of neoplastic cells. Likewise, there was no relationship between size or age of the animals and neoplastic development. The development of the neoplasms could not be associated with any seasonal or cyclic change, nor can we explain the change in the type of neoplasm found at station 5 (1), Long Cove from gonadal to hematopoietic. It is probable that the neoplasms were the result of some alteration in the animals' environment.

Even though the areas were contaminated by oil, we cannot say that the oils were in any way a causative factor in inducing the neoplasms. To determine if the types of oil found in Harpswell Neck (JP-4) and Long Cove (number 2 fuel oil and JP-5) can cause neoplasia in the soft-shell clams, laboratory studies are being conducted at our Environmental Research Laboratory by exposing soft-shell clams to these compounds. So far, the results have been negative.

The blocks and slides examined for this paper are achived at the Registry of Tumors in Lower Animals, National Museum of Natural History, Smithsonian Institution, Washington, D.C., and are available for study.

ACKNOWLEDGMENTS

We thank Dr. Robert Dow, John Hurst, and Frank Ricker, of the Department of Marine Resources, State of Maine, for their excellent cooperation and help with this project.

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